PRODUCTION OF BIOETHANOL FROM TAPIOCA STARCH USING *Saccharomyces serevisiae*: EFFECTS OF pH AND AIR FLOW RATE

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I declare that this thesis entitled "Production of Bioethanol from Tapioca Starch Using *Saccharomyces cerevisiae*: Effects of pH and Air Flow Rate" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature : ………………………………

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Date : 30 April 2009

Special Dedication to my family members,
my friends, my fellow colleague
and all faculty members

For all your care, support and believe in me.

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ABSTRACT

The effects of pH and air flow rate on fermentation process for bioethanol production in 2 L bioreactor using *Saccharomyces cerevisiae* were studied. Other conditions such as temperature, agitation and inoculum concentration were fixed at specific values. Two-step enzymatic hydrolysis of tapioca starch by commercially available α-amylase and glucoamylase were employed at the beginning of the process for glucose production. The cell growth study was conducted in order to observe the cell growth profile in the resulted sugar. The fermentation study was conducted in aerobic condition using 2 L bioreactor with various pHs (4, 5, and 6) and air flow rates (1.0, 1.5 and 2.0 L/min). Based on the results, the optimum pH and air flow rate were pH 5 and 1.0 L/min respectively. The highest yield of ethanol was 3.64 g/L which produced at pH and air flow rate at 1.0 L/min.

ABSTRAK

Kesan pH dan kadar aliran udara terhadap proses penghasilan etanol menggunakan *Saccharomyces cerevisiae* di dalam bioreaktor 2 L telah dikaji. Keadaan lain seperti suhu, pengadukan dan kepekatan inokulum telah ditetapkan pada nilai-nilai tertentu. Proses hidrolisis enzim oleh α-amilase dan glucoamilase digunakan terhadap kanji ubi kayu bagi penghasilan gula. Kajian pertumbuhan sel telah dijalankan bagi mengenal pasti profil pertumbuhan sel pada gula yang dihasilkan. Proses fermentasi telah dijalankan di dalam bioreaktor 2 L pada keadaan aerobik dengan menggunakan pelbagai pH (4, 5, and 6) dan kadar aliran udara (1.0, 1.5 and 2.0 L/min) dijalankan. Berdasarkan data yang diperolehi, keadaan pH dan aliran udara yang optimum adalah masing-masing pH 5 dan 1.0 L/min. Kadar etanol yang tertinggi adalah sebanyak 3.64 g/L yang dihasilkan pada pH 5 menggunakan aliran udara 1.0 L/min.

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LIST OF SYMBOLS/ABBREVIATIONS

Ca^{2+}	-	ion calcium
CO_2	-	carbon dioxide
DNS	-	Di-Nitro Salicylic Acid
Glu.	-	Glucose
g	-	gram
h	-	hour
KNU	-	kilo
mg/L	-	milligram per liter
Mg^{2+}	-	ion magnesium
min	-	minutes
mL	-	mililiter
Mm^3	-	megameter
v/v	-	volume per volume
v/w	-	volume per weight
w/v	-	weight per volume
w/w	-	weight per weight
µg/mL	-	microgram per mililiter
%	-	percentage
°C	-	degree Celsius
°F	-	degree Fahrenheit
µmol	-	micromole

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ethanol (ethyl alcohol, grain alcohol) is a colourless, clear liquid and completely miscible with water. In dilute aqueous solution, ethanol has sweet flavor, but in concentrated form, it has a burning taste, and an ether-like odor. Ethanol is also known as ethyl alcohol (CH_3CH_2OH) or fermentation alcohol. It is referred to a group of chemical compounds, whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. Ethanol has been proposed as a valuable liquid fuel and an alternative to crude oil (Wyman, 2004).

Bioethanol is mainly produced by sugar fermentation process. Industrial ethanol production has utilized using various starchy materials such as corn, wheat, rice or waste straw, potatoes and sago starch as its raw materials. One of the greatest challenges for the 21st century is to meet the growing demand of bioethanol production as energy in sustainable way (Wyman, 2004).

Bioethanol has the potential to provide partial solution to the world's energy whereby it has high octane fuel and able to replace lead as an octane enhancer in petrol. Bioethanol may significantly reduce the amount of imported oil and also allowing large savings in imports costs or increased revenues from export countries (Suraini, 2002).

Blending ethanol with gasoline will oxygenate the fuel mixture caused the fuel to burn completely and reduces polluting emissions (Alexender, 2008).

An agricultural raw material such as rice, wheat and starch is the major carbohydrates suppliers in Malaysia. Tapioca starch has a great potential as an alternative cheap carbon source for fermentation which attract both economic and geographical considerations. In 2003, the world's bioethanol production was 23 Megameter^3 (Berg, 2004). The major world producers, Brazil and United States are together account for about 80% of the world production. The main feedstock for bioethanol production in Brazil is sugarcane while USA utilizes corn grain (Mojović *et al.*, 2006).

Production of bioethanol from starch requires the conversion of polymer starch into glucose at the first place. A fermentable sugar is normally recovered by an enzymatic process that comprises two reaction steps which are liquefaction and saccharafication. Afterward, the process was followed by fermentation process for bioethanol production (Suraini, 2002). α-amylase and glucoamylase are used for enzymatic hydrolysis of starchy materials while *Saccharomyces cerevisiae* yeast is employed in fermentation process (Mojović *et al.*, 2006).

1.2 Objective

The aim of this research is to determine the optimum conditions of few parameters in fermentation process for the production of bioethanol from tapioca starch. Hence, the objectives of this research are:

i. To determine the effect of pH on the production of bioethanol from tapioca starch.
ii. To determine the effect of air flow rate on the production of bioethanol from tapioca starch.

1.3 Scope of Study

Bioethanol production has been conducted by two-step process; enzymatic hydrolysis followed by fermentation process. Various air flow rate and pH in fermentation process were investigated. The bioethanol concentrations produced and glucose consumption were analyzed during the entire study. Other parameters such as enzymatic hydrolysis parameters, fermentation temperature, agitation speed and inoculums concentration were fixed at specific conditions.

1.4 Problems Statement

In 2000, the United States consumed almost 10^{17} BTUs of energy, with almost 40% coming from imported petroleum. High dependence on imported oil would expose to price vulnerability and availability disruption as occurred during "energy crises" on 1970s. Additionally, vehicles miles and total number of vehicles are continued to rise, thus, increased the petroleum demand. Petroleum presents as a finite resource and cannot be sustained indefinitely, and its price will increase over time. Hence, abundant alternative domestic energy sources for transportation are vital (Wyman, 2004).

According to *Oil & Gas Journal* (Malaysian Energy Data, Statistics & Analysis - Oil, Gas, Electricity, 2007), Malaysia held oil reserves of 3.0 billion barrels as of January 2007, down from a peak of 4.6 billion barrels in 1996. Average production for 2006 stood at 798,000 barrels/day decreased by 7 % from 2005's. During 2006, Malaysia consumed an estimated of 515,000 barrels/day of oil while the net exports was about 283,000 barrels/day (Malaysian Energy Data, Statistics & Analysis - Oil, Gas, Electricity, 2007). Malaysia's oil production and consumption during 1998-2008 is given by Figure 1.1.

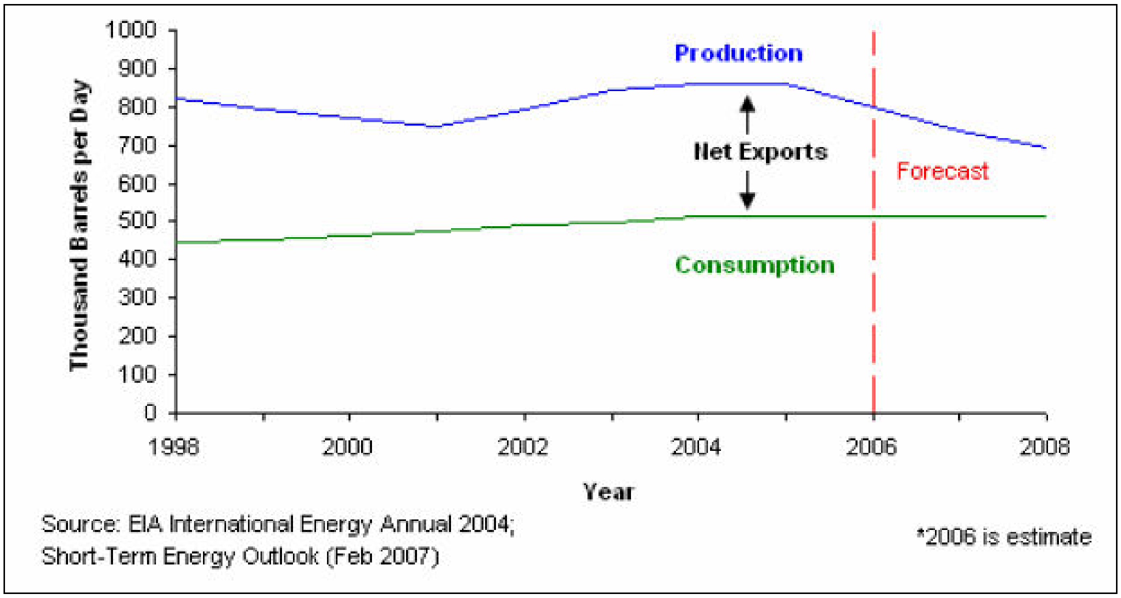

Figure 1.1: Malaysia's Oil Production and Consumption, 1990-2008* (Malaysian Energy Data, Statistics & Analysis - Oil, Gas, Electricity, 2007)

An alternative source for fuel and others petroleum-based products is critically needed. The global demand and unstable conditions in worldwide prices has forced developing country such as Malaysia to re-evaluate their ability to meet their future energy replacements (Malaysian Energy Data, Statistics & Analysis - Oil, Gas, Electricity, 2007).

Bioethanol posed as a valuable liquid fuel alternative to transportation sector. It is one of the most important renewable fuels that contribute to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels. The United States for example has become the largest contributor of greenhouse gases where burning petroleum has caused about 43% of carbon dioxide release into the atmosphere each year. Bioethanol performs as a better transportation fuel as it substantially improves urban air quality and reduces environmental effects. Thus, bioethanol has a great potential and high efficiency for reducing petroleum consumption and practically environmental friendly compared to the occasional fuel from gasoline (Wyman, 2004).

1.5 Rationale and Significance

In Brazil, transportation sector uses over two-thirds of the petroleum consumed; with over 96% of transportation energy comes from this ultimate source. Thus, it will continue on increasing the world petroleum demand. Many other countries also suffer from this similar oil-related strategic which resulted in significant economic difficulties (Wyman, 2004). Therefore, abundant alternative domestic energy sources such as bioethanol would dramatically reduce the total dependent on petroleum. Bioethanol also present as cheap energy source that would be a better alternative fuel compared to occasional fuel. The blending bioethanol with petrol would help to prevent the diminishing of oil supplies and ensure greater fuel security, avoiding heavy reliance on oil producing nations (Prasad *et. al*., 2007).

Bioethanol has a favourable fuel, resource, and environmental attributes as a transportation fuel. In quantities up to 5%, bioethanol can be blended with conventional fuel without the need of engine modifications. Bioethanol is also biodegradable and far less toxic that fossil fuels. Ethanol contains 35% oxygen that helps complete combustion of fuel and thus reduces particulate emission that pose health hazard to living beings. In addition, using bioethanol in older engines can help reduce the amount of carbon monoxide produced by the vehicle thus improving air quality. Thus, bioethanol would give better urban air quality and reduce environment effects of consuming fuels (Chandel *et. al*., 2009).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In the past, fossil fuels were used for transportation and it is totally relied upon the petroleum supply. During the last few decades, the petroleum supply was continuously diminished. There has been a considerable interest in the development of fuels generated from renewable resources, that is to say bio-fuels (Marcos *et. al*, 2007). Ethanol is a favourable fuel and environmental friendly transportation fuel. Ethanol can be made from starch, sugar, abundant, sustainable sources of low-cost cellulosic biomass such as agricultural and forestry residues, portions of municipal waste, herbaceous and woody crops. The transformation of the energy-rich crops such as sugar cane, corn and starch or lignocellulosic biomass requires the pretreatment of the feedstock for fermenting organisms to convert them into ethanol (Cardona, 2007).

The development of cost-effective technologies for fuel ethanol production is a priority for many research centers, universities, private firms, and even for governments. Process engineering applied into the production of bioethanol includes the design of new innovative process configurations, aimed at reducing ethanol production costs. On the other hand, the development of environmental friendly technologies for bioethanol production can be carried out by utilizing the different design approaches. Particularly, the production of bioethanol gives lower impact on global climate change of the greenhouse gas emissions. Ethanol has been trusted as an alternate fuel for the future and is already produced on a fair scale (about 14-26 million tons) worldwide. The bulk of the production is located in Brazil (16 billion liters produced in 2005) and the USA (10.6 billion liters in 2003) (Hamelinck *et al*. 2005). Bioethanol is expected to be one of the dominating renewable biofuels in the transportation sector within the next 20 years (Hägerdal *et al*. 2006).

2.2 Background of Ethanol

Ethanol is known as ethyl alcohol or fermentation alcohol. Ethanol is a member of the alcohol family which has a chemical formula C_2H_5OH. It is a monohydric primary alcohol. Ethanol melts at -117.3°C and boils at 78.5°C. It is miscible with water in all proportions and is separated from water only with difficulty. Other than chemically produced from ethylene, ethanol can be produced from starch, sugar crops such as sugar-cane, maize, sorghum, and wheat and other grains, or even cornstalks, fruit and vegetable waste. Ethanol also can be made from cellulosic biomass such as agricultural residues, industrial waste, herbaceous plants and portions of municipal waste. Ethanol is used extensively as a solvent in the manufacturing of varnishes and perfumes, essences and flavorings, medicines and drugs, and as a fuel and gasoline additives (Wyman, 2004).

2.3 Ethanol Production from Sugar Fermentation

Ethanol can be produced by two routes; (1) fermentation of sugars derived from sugar, starch, or cellulosic materials or (2) reaction of ethylene with water. Production of ethanol from sugar required microbial fermentation. The fermentable raw materials can be categorized as fermentable sugary materials, starchy or lignocellulosic materials. Starch and cellulose components in those raw material can be transformed into sugars specially glucose that be used as fermentation substrates (Equation 2.1). In sugar fermentation, the phosphorylation of carbohydrates is carried out through the metabolic pathway and the end products are two moles of ethanol and carbon dioxide (Prasad *et. al*, 2007). Two moles of ethanol and CO_2 were produced for every mole of glucose consumed (Equation 2.2).

$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6$ (Equation 2.1)

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ +energy (stored as ATP) (Equation 2.2)

Since the yeast cannot use starch directly for ethanol production, starch has to be wholly convert into glucose where two known enzymes, which are α-amylase and glucoamylase are basically used. Hence, ethanol production from starch involves enzymatic hydrolysis to release fermentable sugar, followed by fermentation with yeast (Prasad *et. al*, 2007).

2.4 Raw Materials or Feedstock for Ethanol Fermentation

2.4.1 Sugar

Direct sugar feedstock for bioethanol is essentially comprised of sugar cane and sugar beet. Direct fermentation of sugarcane, sugar beet and sweet sorghum to produce ethanol has been widely reported. Sugar containing materials required the least costly pretreatment, whereas starchy, lignocellulosic materials and industrial wastes needed prior pretreatment to convert into fermentable substrates (Prasad *et. al*, 2007).

2.4.2 Starch

Another type of feedstock which can be used for bioethanol production is starch-based materials. Starch is a biopolymer consisting only one monomer, D-glucose. To produce bioethanol from starch, it is necessary to break down the chains of this carbohydrate for obtaining glucose, which can be converted into bioethanol by yeasts (Figure 2.1). This is the most utilized feedstock for bioethanol production in North America and Europe where corn and wheat are mainly employed (Balat *et.al*, 2008).

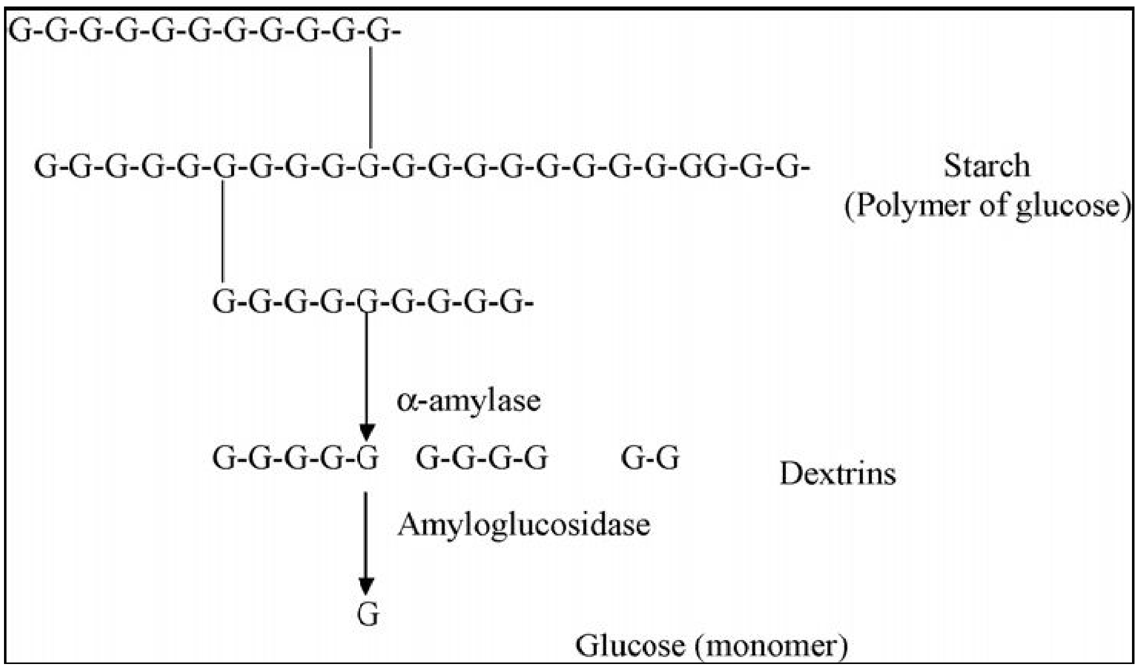

Figure 2.1: Scheme of an enzymatic hydrolysis of starch to glucose (Prasad *et.al*, 2007).

Starch is the major dietary source of carbohydrates, and the most abundant storage polysaccharide in plants, occurring as granules of size 1 to 100 μm (Phillips and Williams, 2000). Starch is composed of a mixture of two kinds of polyglucans, namely amylose and amylopectin. The structure of amylose and amylopectin is shown in Figure 2.2. Starch is the most essential component in most of the food preparation and also the major source of calories in the tropics.

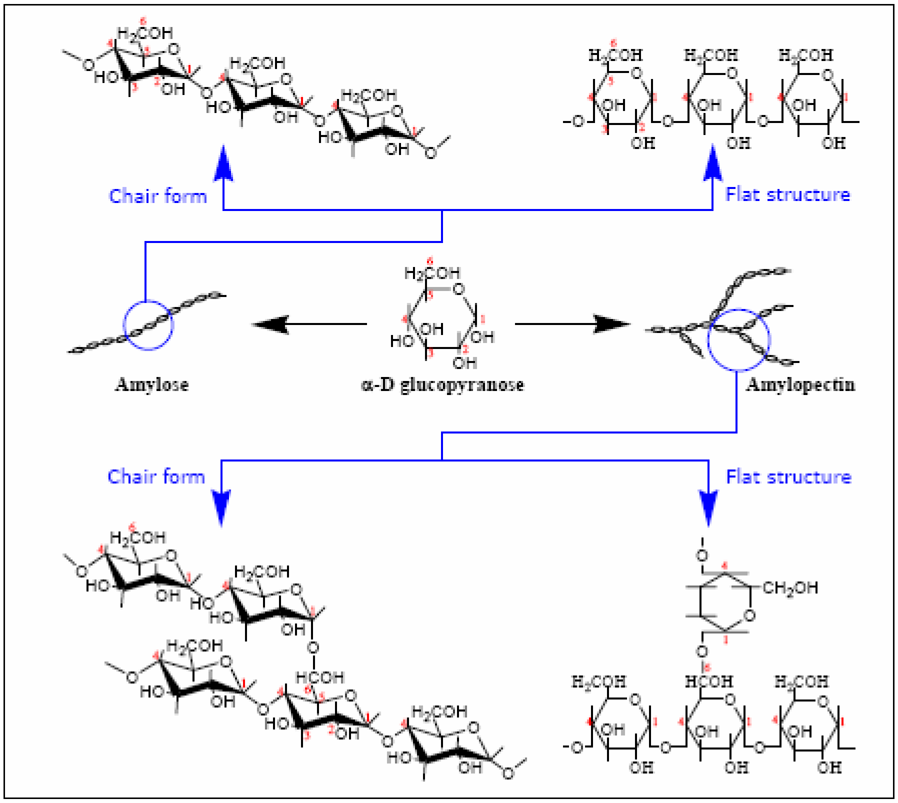

Figure 2.2: Scheme representing the linear and branched starch building polymers. (Marcos *et. al.*, 2007).

Industrial ethanol production has been reported using various starchy materials such as starch, corn, wheat, potatoes, and cassava root. Cassava (*Manihot esculenta*) is a perennial woody shrub with up to 32% of starch content (Nigam and Singh, 1995). Tapioca starch is a potential raw materials which rich in fermentable carbohydrates. It is abundantly found in Malaysia and the prices are cheaper compared to the starchy-based materials. Nutritional value of the cassava tubers is given by Table 2.1.